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13. ABSTRACT (Maximum 200 words)

Microbial control of biodegradative enzymes generally is exercised at the level of transcription. Thus an understanding of existing pathways for biodegradation demands knowledge, not only of the enzymes, but also of the mechanisms that control their synthesis. In this research program we have explored the properties of an evolutionarily related set of genes that control transcription of catabolic enzymes. Despite their similarities, the regulatory genes exert highly specific controls at the level of DNA. Using newly developed procedures based on random polymerase chain reaction (PCR) mutagenesis, we have been able to select mutants in which one of the transcriptional activators has been inactivated by a wide range of amino acid substitutions. This advance has established a basis for analysis of how the primary structure of the activator protein influences its activity. Furthermore, we have shown that relatively minor genetic modification is sufficient to alter one regulatory gene so that it can assume the function of an evolutionarily related regulatory gene that had been deleted from the chromosome.

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Final Progress Report

Evolution of Regulatory Genes Governing Catabolic Pathways in *Acinetobacter*

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Biodegradation of complex chemicals requires the coordinated activities of numerous enzymes. Microbial control of such enzymes generally is exercised at the level of transcription. Thus an understanding of existing pathways for biodegradation demands knowledge, not only of the enzymes, but also of the mechanisms that control their synthesis. In this research program we have explored the properties of an evolutionarily related set of genes that control transcription of catabolic enzymes. Despite their similarities, the regulatory genes exert highly specific controls at the level of DNA. Nevertheless, relatively minor genetic modification is sufficient to alter one regulatory gene so that it can assume the function of another regulatory gene that has been deleted from the chromosome.

The first of the regulatory genes to be elucidated was *pobR* from an *Acinetobacter* strain that is unique among bacteria in its extraordinary competence for natural transformation. This unusual genetic property has greatly facilitated both analysis and manipulation of genes governing biodegradation. Mutations inactivating *pobR* prevent the inducible expression of *pobA*, structural gene for the hydroxylase that converts *p*-hydroxybenzoate to protocatechuate. Organisms containing such mutations are readily selected in strains containing a mutation that blocks protocatechuate catabolism at the level of a toxic metabolite.

Genetic and biochemical analysis demonstrated that *pobR* and *pobA* are divergently transcribed and physically separated by a 134 bp intergenic region containing an operator to which PobR binds. This DNA is characterized by a 40 bp segment containing direct repetitions of a 9 bp signature sequence (TGTCCGATG) followed by 9 bp of apparently unrelated sequence directly preceding an inversion of the 9 bp signature sequence. As we have learned through this investigation and the work of others, variants of this operator sequence govern transcription of different genes associated with catabolism of aromatic acids.

Spontaneous mutations blocking *pobR* are unusual in that they tend to be caused by disruption introduced by the newly discovered insertion sequence IS*1236*. These mutations do not occur at a single hotspot but rather are distributed throughout the gene. Thus it appears that the insertions are not determined so much by sequence as by another property of *pobR* DNA, perhaps its topology.

DNA altered by IS*1236* is increased in length by 1.2 kb and therefore is easily identified by Polymerase Chain Reaction (PCR). Therefore the background of spontaneous mutation in *pobR* offered a system in which a novel technique for recovery of mutants containing random mutations generated within a targeted region of DNA by PCR replication errors during amplification. Primers on either side of the target (in this case *pobR*) are used to amplify it, and the amplified DNA serves as donors for recipient strains in which the function of the targeted DNA prevents growth. Transformation with *Taq*-amplified DNA *pobR* DNA increased the mutations frequency 240-fold and reduced the level of *IS*1236 inserts to undetectable levels. Sequence analysis of 89 of the mutant *pobR* alleles showed that the mutations were predominantly single-nucleotide substitutions broadly distributed within *pobR*. Promoter mutations were recovered, as were two mutations likely to block *pobR* translation. One third of the recovered mutations conferred a leaky or temperature-sensitive phenotype. Therefore the general application of this procedure is likely to afford a sensitive assessment of mutations causing partially impaired function that can be detected at the level of phenotype.

A separate study led to characterization of PcaU, the transcriptional activator of the *pca* operon which encodes enzymes for dissimilation of protocatechuate. The *pcaU* regulatory system was discovered by sequencing of DNA flanking a mutation designated *pcaP1* now known to be a regulatory mutation that blocks transcription of the *pca* operon of structural genes. This operon is separated from the divergently transcribed *pcaU* by 282 bp of DNA containing both *pcaP1* and a 40 bp DNA segment that closely resembles the *pobR* operator. PcaU and PobR share share common ancestry as indicated by 54% identity of their amino acid sequences. Characterization of *pcaU* was delayed by the fact that knockout mutations in this gene prevent neither expression of the *pca* operon nor growth of the bacteria with protocatechuate at 37°, the temperature at which this strain of *Acinetobacter* is conventionally grown in the laboratory. Exposure of *pcaU* knockout strains to 22° does prevent their growth with protocatechuate, so this temperature was used for genetic characterization of the *pca* regulatory system. This work is still in progress, but it is notable that single nucleotide substitutions in either *pcaU* or *pobR* were sufficient to alter the product of the one gene so that it could complement knockout mutations in the other gene. Thus very little genetic information is required to achieve the extraordinary specificity that allows cells to distinguish between *p*-hydroxybenzoate (4-hydroxybenzoate) and protocatechuate (3,4-dihydroxybenzoate) as modulators effecting transcriptional control.

Important components of regulatory systems are the proteins that govern transport of potential growth substrates into the cell. Sequencing has revealed genes for multiple transport systems, and the available evidence indicates that these systems have overlapping substrate specificities. The physiological functions of the transport systems are being established by characterization of the phenotypes of strains in which the transport genes have been inactivated by mutation.

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Publications

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Gerischer, U., A. Segura, and L. N. Ornston. 1998. PcaU, transcriptional activator of genes for protocatechuate utilization in *Acinetobacter*. J. Bacteriol. In press.

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